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14. ABSTRACT

To test the idea that ovarian cancer arises from oviductal epithelium, spreads to the ovary, and presents as ovarian cancer, we generated a mouse model in which we can target genetic deletions to the oviductal epithelium. We are currently performing selective genetic deletion of ovarian cancer genes, such as TP53, Rb, and BRCA1 in oviductal epithelium in this mouse model.

15. SUBJECT TERMS
Mouse models, fallopian epithelium, oviductal epithelium, BRCA1, Rb, TP53

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INTRODUCTION: In addition to previously accepted idea that epithelial ovarian cancer arises from surface epithelium of the ovary, recent evidence suggests that high-grade serous subtype of epithelial ovarian cancer may also arise from the fallopian epithelium. This shift in cell-of-origin for ovarian cancer has profound implication in the diagnosis, screen, and the pathobiology of ovarian cancer. However, appropriate mouse models to test a paradigm-shifting hypothesis that high-grade serous ovarian cancer may arise from fallopian tubal epithelium are lacking. Therefore, in this proposal we generated a mouse model to test the hypothesis that tubal epithelium could serve as an original site for ovarian cancer.

BODY: In the approved SOW for Year 1, we proposed to continue the development of mouse models for tubal carcinogenesis. There are two concurrent specific aims on Year 1 of Statement of Work.

Specific Aim 1 (1-24 months): To generate mouse models of tubal carcinogenesis

We have accomplished the following tasks:

- Generated a mouse model targeting expression of Cre-ERT2 to the fallopian tube using Ovgp1 promoter (Figure 1).
- Generated floxed p53/Rb and floxed p53/BRCA1 mice (Figure 2)
- Generating floxed p53/Rb; Ovgp1::Cre-ERT2 triple transgenic mice and floxed p53/BRCA1; Ovgp1::Cre-ERT2 triple transgenic mice
- Established immunohistochemical staining conditions for p53, Ki67, WT1, PAX2, PAX8, and cytokeratin (Figure 3 and Table 1)

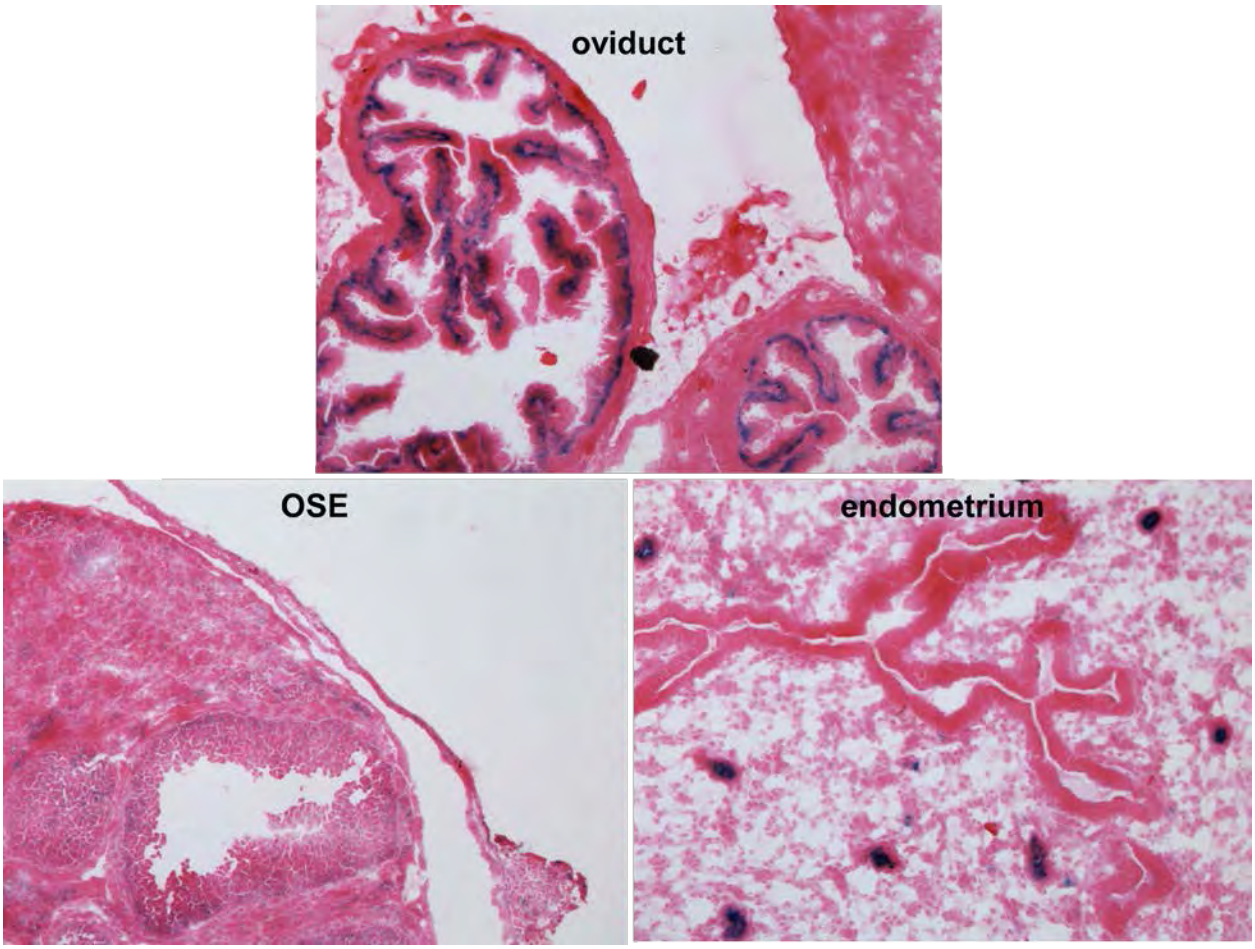

Figure 1. Generation of Ovgp1::Cre-ERT2 mice. After the mice are generated, tissue-specific expression of Cre is tested by crossing the mice with ROSA26 reporter mice. Upon recombination induced by tamoxifen, oviductal tissues are positive for b-gal staining, and ovarian surface epithelium and the ovary is negative for b-gal staining. Endometrial epithelium showed positive staining for b-gal.

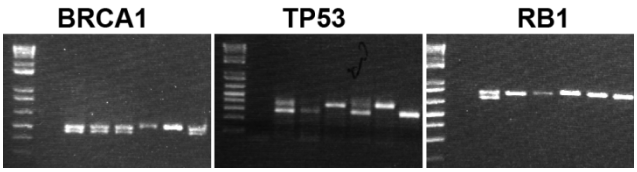

Figure 2. Generation of floxed transgenic mice. To generate the double and triple genetic mice (TP53/BRCA1; Ovgp1::Cre-ERT2 and TP53/Rb1; Ovgp1::Cre-ERT2), we purchased these mice from the National Cancer Insitute, and generated double transgenic mice by crossing BRCA1 mice with TP53 mice or Rb1 mice with TP53 mice. These mice carried floxed alleles, which can be distinguished from wild-type allele by presence of slower migrating PCR amplicons.

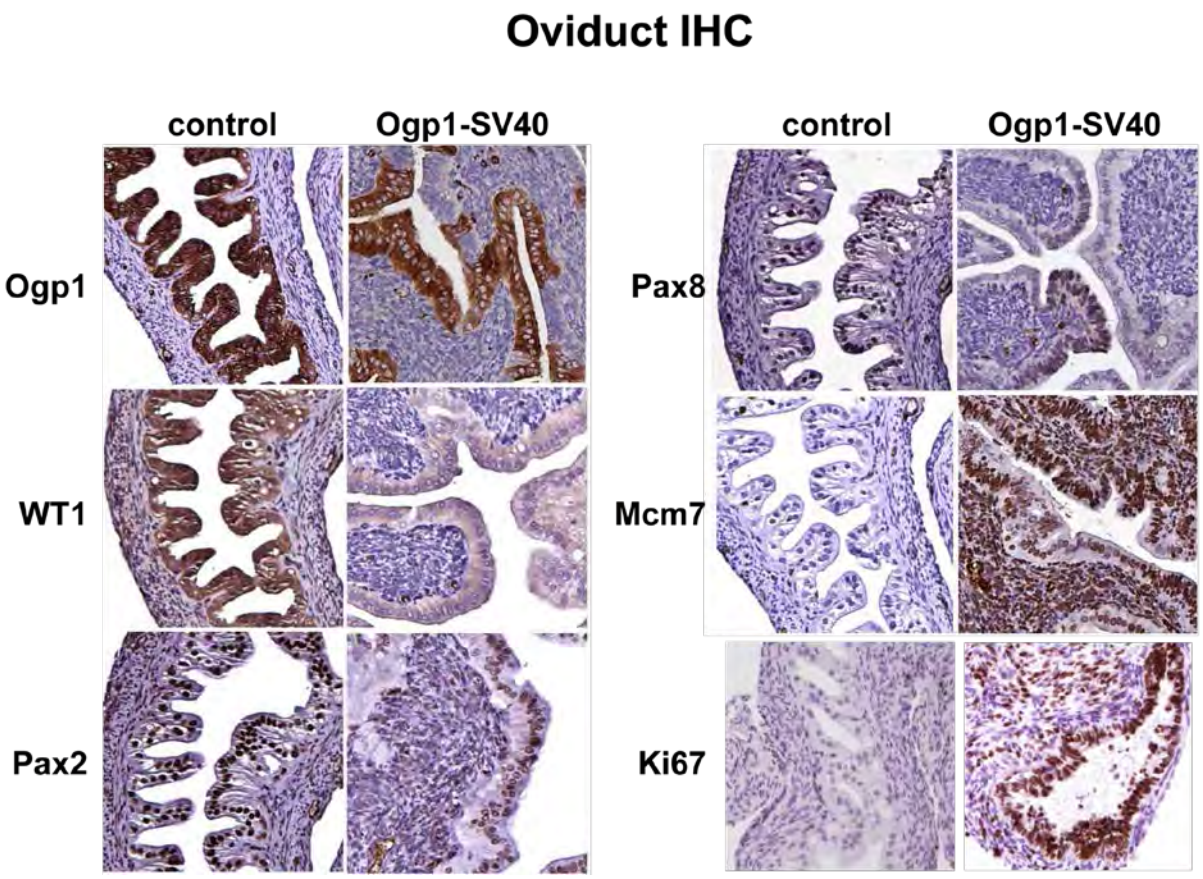

Figure 3. Immunohistochemical analysis of WT1, Pax2, Ovgp1, Pax8, MCM7 and Ki67 markers in mouse tumor samples.

Summary of the staining

	oviduct		endometrium	
	control	Ogp1-SV40	control	Ogp1-SV40
SV40	-	+++	-	++
p53	-	+	-	+
Ogp1	++	+, patchy	-	-
WT1	++	-	+	++
Pax2	++	±	++	+
Pax8	++	+, patchy	-	-
Mcm7	-	+++	+	++
Ki67	-	+++	±	+

Table 1. Summary of immunohistochemical staining patterns of select proteins in the oviduct and endometrium of normal and transgenic mice.

Specific Aim 2 (1-24 months): To characterize tumor initiations and progression in conditional double knock-out of p53/Rb and p53/BRCA1 mice

- Concurrent with Specific Aim 1, we are generating triple transgenic mice to monitor tumor development at various time points. These experiments will continue with the approval by ACURO required as part of the institutional transfer.

KEY RESEARCH ACCOMPLISHMENTS:

- Generated a mouse model targeting expression of Cre-ERT2 to the fallopian tube using Ovgp1 promoter (Figure 1).
- Generated floxed p53/Rb and floxed p53/BRCA1 mice (Figure 2)
- Generating floxed p53/Rb; Ovgp1::Cre-ERT2 triple transgenic mice and floxed p53/BRCA1; Ovgp1::Cre-ERT2 triple transgenic mice (Figure 3)
- Established immunohistochemical staining conditions for p53, Ki67, WT1, PAX2, PAX8, and cytokeratin (Figure 4)

REPORTABLE OUTCOME: Studies are ongoing, and interim analysis of data indicates that we were able to generate triple transgenic mice. In addition, we established conditions optimal for induction of genetic recombination mediated by Cre under the control of tamoxifen. The interim results of the study were presented at the 2012 Annual Meeting of the American Association for Cancer Research. In addition, this funding has resulted in the employment of 0.5 FTE for a research fellow.

CONCLUSION: The study period starts from the August 2011 to August 2012. During that period, we started with planned studies by acquiring the transgenic mice needed for the study and by setting up mating cages for generating double and triple transgenic mice. In addition, we started establishing immunohistochemical staining procedures for expression of select proteins in mouse tissues and tumors. We established PCR conditions for genotyping of transgenic mice. In April of 2012, I moved from Mayo Clinic and transferred to current institution, University of Kansas Medical Center. As part of the move, the mouse research program was shut down as of April 13, 2012. We plan to restart the research studies as soon as new approved protocol is reviewed by ACURO. We have obtained approval from local IACUC for the resumption of studies. We have submitted the new protocol to be reviewed by ACURO.